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Experimental evidence that kin discrimination in the Seychelles warbler is based on association and not on genetic relatedness

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In cooperative breeding systems driven by kin selection, effective kin-recognition cues are important. Recognition could be achieved by the direct assessment of the genetic relatedness of individuals or by learning through association. In the Seychelles warbler, *Acrocephalus sechellensis*, female subordinates maximize indirect fitness by preferentially helping genetically related nestlings. Help seems to be based on the continued presence of the primary female who previously fed the subordinate in the nest but it has, so far, been impossible to discount the direct assessment of genetic relatedness. We used a cross-fostering experiment to separate the two possible cues. Adult birds did not discriminate between their own and cross-fostered eggs or nestlings. Cross-fostering resulted in nestlings that were unrelated to the primary female that raised them, but control nestlings were closely related to their primary females. The proportions of cross-fostered and control female offspring that stayed and became helpers on their 'natal' territory were similar. However, for both groups the chance of becoming a subordinate helper was associated with the continued presence of the primary female and not with any other factor tested. Our study provides strong evidence that helping decisions are based on associative-learning cues.

Keywords: kin discrimination; genetic relatedness; cooperative breeding; Seychelles warbler; kin recognition; associative learning

1. INTRODUCTION

Cooperative breeding is a reproductive system where some individuals become subordinates within a group and assist the breeding pair in rearing offspring (Brown 1987). In most cooperatively breeding species, subordinates are constrained from breeding independently, but by becoming subordinates and helping they improve the reproductive success and/or survival of the recipients (Emlen 1997). Theoretical studies have shown that cooperation with close relatives increases the indirect fitness gains accrued by subordinates and have suggested that kin selection is a driving force behind the evolution of cooperative breeding systems (Hamilton 1964; Maynard Smith 1964). If so, the ability of subordinates to discriminate between kin and non-kin will be important in maximizing their fitness. Studies on subordinate investment in species where within-group relatedness between individuals varies have provided mixed evidence for kin preferences (see Griffin & West 2003). Some studies have shown no increased investment by subordinates with respect to kinship (Cockburn 1998; Clutton-Brock *et al.* 2000, 2001). Others have shown facultative bias of helping by subordinates towards close kin (based on genealogical data (Clarke 1984; Reyer 1984; Curry 1988; Emlen & Wrege 1988; Arnold 1990; Komdeur 1994*a*; Russell 1999)), or towards more genetically related birds (Russell & Hatchwell 2001; Richardson *et al.* 2003*a*,*b*).

Subordinates could potentially assess kinship through a variety of mechanisms (reviewed in Komdeur & Hatchwell 1999). Recognition could be achieved directly, by phenotype matching of unfamiliar individuals to some phenotypic-recognition template acquired through genetic encoding (Sherman *et al.* 1997; Petrie *et al.* 1999; Hauber & Sherman 2001, 2003). Alternatively, kin recognition could be achieved indirectly by learning through association, if there is a reliable correlation between genetic relatedness and association (Blaustein *et al.* 1987). Several studies have investigated kin-recognition cues in cooperative bird species (Payne *et al.* 1988; Komdeur 1994*a*; Price 1999; Russell & Hatchwell 2001; Richardson *et al.* 2003*a*,*b*), but only one study, on the long-tailed tit, *Aegithalos caudatus*, has investigated experimentally the mechanism through which kin discrimination works (Hatchwell *et al.* 2001). In this species, most helpers are brothers of the male breeder whose brood they feed. When given the choice between broods belonging to kin and those belonging to non-kin, helpers preferentially helped kin (Russell & Hatchwell 2001) and appeared to accomplish this by discriminating between their vocalizations. Partial cross-fostering of nestlings showed that cross-fostered siblings did not discriminate between related and unrelated brood mates, indicating that the recognition mechanism involves learning their siblings' vocalizations through association (Hatchwell *et al.* 2001).

In contrast to helpers in the long-tailed tit, subordinate helpers in the Seychelles warbler, *Acrocephalus sechellensis*,

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mainly provision at the nests of their parents and are more likely to provision when related to the nestling (Komdeur 1994*a*; Richardson *et al.* 2003*a*,*b*). Subordinate helpers significantly improve the reproductive success of the breeding pair (Komdeur 1994*b*; Richardson *et al.* 2003*a*) and also increase their own indirect fitness benefits by preferentially helping genetically related nestlings (Richardson *et al.* 2003*a*,*b*). Effective kin-recognition cues are, therefore, important in this species. The life-history parameters of the Seychelles warbler provide a situation in which kin recognition through association may develop. A breeding pair remains together within a territory as long as both birds survive, and offspring have a long period of dependence, remaining on the natal territory for at least 1 year (Komdeur 1992). Evidence that kin discrimination in this species is achieved through association, and not by direct assessment of relatedness, is twofold: (i) when a subordinate has a choice of helping at two different nests on their natal territory, it does not always feed the nestling to whom it is most related; instead it feeds only at those nests with breeders that fed it as a nestling (Komdeur 1994*a*); and (ii) the decision to help a breeding pair appears to be based on the continued presence of the primary female (but not the male) who previously fed the subordinate in the nest (Richardson *et al.* 2003*a*,*b*). The female's continued presence predicted the subordinate's provisioning behaviour better than did subordinate–primary female or subordinate–nestling relatedness but, as the continued presence of the primary female reliably indicated relatedness to the nestling (Richardson *et al.* 2003*a*,*b*), this cue was still effective in maximizing indirect benefits to subordinates. The primary male was not used as a cue, which is logical in an evolutionary sense, as a high frequency of female infidelity (Richardson *et al.* 2001) leads to subordinates often being unrelated to the primary male. Consequently, his continued presence did not reliably indicate relatedness between the subordinate and the nestling (Richardson *et al.* 2003*a*,*b*). However, these data were only correlative. To unravel the mechanisms of kin recognition adequately, experiments are required (Komdeur & Hatchwell 1999).

The present study uses the cross-fostering of eggs or young nestlings to determine experimentally whether subordinates assess their genetic relatedness to the primary female directly or by association. We use genotypic data from microsatellite markers to determine the genetic relatedness between individuals. We predict that if provisioning is based upon an associative-learning cue then cross-fostered young will stay on the 'natal' territory as subordinates and become helpers at nests belonging to the unrelated primary females who fed them when they were nestlings, and that the probabilities of staying on the territory and helping are equal for cross-fostered and non-cross-fostered young. However, if genetic relatedness is assessed directly then cross-fostered young will not stay on the 'natal' territory and help the primary female who fed them.

2. METHODS

(a) *Study species and data collection*

The Seychelles warbler is a passerine bird endemic to the Seychelles islands in the western Indian Ocean. The Cousin Island (28 ha) population of Seychelles warblers has been monitored intensively since December 1985. During this time, almost all birds have been individually colour-ringed (using a unique combination of three ultraviolet-resistant colour rings and a British Trust for Ornithology metal ring) and monitored throughout every breeding season (June to September). Therefore the reproductive history, putative pedigree and status of the majority of the Seychelles warblers are known. Although warblers can breed independently in their first year, a lack of suitable independent breeding opportunities drives many individuals (62%) into becoming subordinates within their natal territory (Komdeur 1992).

The present study was based on adult warblers observed during the main breeding season on Cousin Island in the years 1995–2002. Approximately 96% of birds were colour-ringed and blood sampled. Blood samples (*ca*. 15 µl) were collected by brachial venepuncture, diluted in 800 µl of 100% ethanol in a screw-cap microfuge tube and stored at room temperature. The identities of all colour-ringed birds present in each territory were recorded. The statuses of all adult birds were based upon field observations combined with the available long-term demographic data. The 'primary' male and female were defined as the dominant pair-bonded male and female in the territory, while the term 'subordinate' included all other adult birds resident in the territory (Richardson *et al.* 2002). Subordinates within a territory do not always help (Richardson *et al*. 2003*a*,*b*) and were defined as 'helpers' only if they had been observed incubating or provisioning at a nest, in the territory, in a given year. All territories were checked fortnightly for nesting by following the primary female for 15 min (Komdeur 1992). Most nests were checked for clutch and brood size. The Seychelles warbler produces one clutch per season and this normally consists of just one egg (80%); however, on occasion two- or three-egg clutches may occur (Komdeur 1991; Richardson *et al.* 2001).

(b) *Cross-fostering experiment*

Cross-fostering was conducted during the main breeding season (June to September) in all years from 1995 to 1999. Ideally, cross-fostering should be conducted on eggs to avoid any possibility of the young imprinting on the parents at the original nest. However, to avoid the risk of damaging the egg, in later years we delayed cross-fostering until the early nestling stage. Each territory contained a single nest at any given time. Nests built in the same territory in different years were used only if the identities of both primary birds differed. In total, 100 pairs of nests (including control nests) from 89 territories were selected, such that each pair of nests had eggs of approximately the same laying date (maximum age difference of less than 3 days) or nestlings of the same hatching date (maximum age difference of less than 2 days).

Out of the 27 pairs of nests containing an egg each, 13 were used for cross-fostering (five in 1995; eight in 1996) and 14 as control nests (five in 1995; nine in 1996). Eggs were switched in the second week of incubation (14–16 days incubation period). During the egg cross-fostering procedure a replacement dummy egg, which is readily accepted and incubated by Seychelles warblers (Komdeur 1999), was put in the nest. Out of the 73 pairs of nests containing nestlings (nestling dataset), 38 were used for cross-fostering (three in 1995; four in 1996; eight in 1997; 12 in 1998; 11 in 1999) and 35 as controls (three in 1995; four in 1996; seven in 1997; 11 in 1998; 10 in 1999). To control for the potential effects of date and year, the cross-fostering of eggs

or nestlings in most cross-fostered nests (92.2%, 47 out of 51) was conducted within 3 days of the sham shifting of eggs or nestlings in control nests. Nestlings were switched before the sixth day of age (a nestling stays in the nest for 18–20 days). In control nests the egg or nestling present was removed and put back in the same nest after a short interval (removal times: experimental nests (12 eggs and 16 nestlings combined) 15.9 ± 7.4 min and control nests (10 eggs and 24 nestlings combined) 12.9 ± 1.2 min; $t = 1.66$, d.f. = 60, $p = 0.102$). All nestlings were blood sampled during their removal. The 190 nestlings (including the 44 nestlings that hatched from control and experimental eggs (10 did not hatch) and the 146 (38×2 experimental and 35×2 control) nestlings from the later cross-fostering events) are referred to as the combined dataset. Egg and offspring mortality in the first year is high in the Seychelles warbler (Komdeur 1992; Komdeur & Kats 1999) and, in total, only 13 experimental and seven control eggs resulted in nestlings that survived to 1 year of age, while 30 experimental and 31 control nestlings survived to 1 year of age. The survival, helping and dispersal behaviours of all birds (cross-fostered and non-cross-fostered offspring) were monitored in subsequent years.

In 1995 and 1996, food provisioning was monitored for two 90 min periods at each of a random subset of 33 experimental nests to determine whether the cross-fostering procedure itself affected provisioning. Provisioning was assessed once in the morning prior to the swap and once during the morning of the next day (20 nests with a breeding pair, and 13 nests with a breeding pair and one female subordinate). The provisioning frequency per nest (feeds per hour)—identical to provisioning frequency per nestling because each nest contained one nestling—was calculated for each bird in the territory.

(c) *Molecular methods*

DNA extraction, genotyping and parentage analysis were completed following the methodology described in Richardson *et al.* (2001). The software program KINSHIP was used to calculate individual pairwise relatedness values (r) based on microsatellite genotype similarity at 14 loci isolated in the Seychelles warbler (Richardson *et al.* 2000). The mean pairwise relatedness between two individuals selected at random in the population equalled zero. As not all individuals present in the population before 1997 were genotyped, we were able to calculate pairwise subordinate–primary female relatednesses for only a subset of subordinates. Molecular sexing was performed using the PCR method devised by Griffiths *et al.* (1998) to confirm the sex of each individual.

(d) *Statistical analyses*

Only those birds that had an opportunity to help their putative parents (cross-fostered or not) were included in the analyses of helping decisions, i.e. those with a nest containing young in their 'natal' territory. Binary logistic regression analysis was used to test the effects of multiple independent terms on the survival and provisioning behaviour of offspring. The following factors were included in the analysis of provisioning, as these have previously been shown to influence helping behaviour: territory quality (determined following Komdeur (1994*a*)), subordinate sex, number of offspring, group size (primary and subordinate birds) (Komdeur 1991, 1994*b*) and the continued presence of the primary female (Richardson *et al.* 2003*a*,*b*). The influence of subordinate age on provisioning behaviour (Komdeur 1994*b*) was controlled for by considering subordinates only during their second year of age (only a few subordinates stayed on their natal territory during their third year of age; see § 3b). The stage of experimentation (during the egg stage or nestling stage) and the continued presence of the primary male were also included. Potential explanatory terms were fitted using the stepwise backwards removal of non-significant terms. Year of breeding, subordinate sex and cross-fostering were entered as categorical terms; all other terms were entered as variates. Tables 1 and 2 show the terms removed from the model, their predicted effect (for terms remaining in the model) and their level of significance. The Wald test statistic is given. Statistical analyses were performed using SPSS v. 11.0 (SPSS 2001) and were two-tailed. Mean values are expressed with standard errors; probability values are two-tailed and the level of significance was set at $p < 0.05$.

Table 1. Logistic regression analyses of the effects of territory quality, year of handling, cross-fostering, group size, stage of experimentation (egg or nestling) and nestling sex on the survival of nestlings to 1 year of age (combined dataset, $n = 190$). (Terms highlighted in bold were left in the minimal adequate model after stepwise removal of non-significant variables.)

explanatory terms	Wald	d.f.	p
territory quality	**29.935**	**1**	**< 0.001**
year	**14.816**	**4**	**0.005**
cross-fostering	0.361	1	0.548
group size	0.241	1	0.623
experimentation stage	0.001	1	0.992
nestling sex	0.001	1	0.992

3. RESULTS

(a) *No discrimination between cross-fostered and sham-switched eggs or nestlings*

The mean survival of cross-fostered eggs to hatching was not different from that of sham-switched eggs in control nests (experimental nests: 81% (21 out of 26); control nests: 82% (23 out of 28); $\chi^2 = 0.02$, d.f. = 1, $p = 0.897$). Using the combined dataset of all cross-fostered and control nestlings ($n = 190$), we found that cross-fostering, group size, nestling sex and the stage of experimentation (egg or nestling) did not affect first-year survival (table 1; mean first-year survival of cross-fostered nestlings: 44% (43 out of 97); sham-switched nestlings: 41% (38 out of 93)). There was a significant positive relationship between territory quality and survival to 1 year of age (table 1). The year of handling also had a significant effect on first-year survival.

It is possible to identify which recognition mechanism is used—associative learning or the direct assessment of relatedness—only because of two factors.

(i) Birds did not discriminate between their own and fostered nestlings. At experimental nests, provisioning by primary and female subordinate birds to their own nestling before the swap did not differ from that to the foster nestling after the swap (feeds per hour: primary male: 8.6 ± 0.5 versus 8.2 ± 0.5, paired t-test: $t = 0.54$, d.f. = 32, $p = 0.59$; primary female: 9.1 ± 0.7 versus 9.9 ± 0.8, $t = 0.85$, d.f. = 32, $p = 0.40$; female subordinate: 3.5 ± 0.9 versus 4.3 ± 0.9, $t = 1.49$, d.f. = 12, $p = 0.16$).

Table 2. Variables predicting the helping activity of female subordinate Seychelles warblers during their second year of life. (*a*) Variables without subordinate–primary female relatedness ($n = 38$). (*b*) Variables with subordinate–primary female relatedness ($n = 26$).
(Terms highlighted in bold were left in the minimal adequate model after stepwise removal of non-significant variables.)

explanatory terms	Wald	d.f.	*p*
(*a*)			
presence of primary female	**6.413**	**1**	**0.005**
group size	−2.934	1	0.184
year	7.416	4	0.178
territory quality	0.814	1	0.509
cross-fostering	0.907	1	0.254
experimentation stage	0.793	1	0.530
presence of primary male	0.802	1	0.388
(*b*)			
presence of primary female	**5.515**	**1**	**0.019**
subordinate–primary female relatedness	0.006	1	0.938
group size	−2.661	1	0.103
year	6.443	4	0.168
territory quality	0.778	1	0.378
cross-fostering	0.105	1	0.746
experimentation stage	1.377	1	0.241
presence of primary male	0.598	1	0.439

Therefore the strength of the recognition template based on the primary bird feeding the nestling was identical for cross-fostered and control nestlings.

(ii) Cross-fostered nestling–primary female relatedness was no higher than that between random individuals within the population ($r = 0.05 \pm 0.04$ versus zero; one-sample *t*-test: $t = 1.07$, d.f. = 26, $p = 0.30$), but there was significant control nestling–primary female relatedness ($r = 0.38 \pm 0.04$ versus zero; one-sample *t*-test: $t = 8.50$, d.f. = 27, $p < 0.001$). Cross-fostered nestling–primary female relatedness was significantly lower than control nestling–primary female relatedness ($t = 5.30$, d.f. = 53, $p < 0.001$). Thus the strength of the relatedness template was lower for cross-fostered nestlings than for control nestlings.

(b) *Helping decisions: associative learning or kin recognition?*

Out of the 81 nestlings (cross-fostered and control) that survived their first year, 78 had an opportunity to help at a nest within their 'natal' territory in their second year. Consistent with previous studies (Komdeur 1996*a*), 90% of helpers were females. As the number of male helping subordinates was limited, and only female subordinates appear to adjust provisioning according to relatedness (Richardson *et al.* 2003*a*,*b*), all further analysis was restricted to female subordinates ($n = 38$).

The continued presence of the primary female explained most of the variance in the fraction of female subordinate birds staying and helping during their second year of age, both for control and for cross-fostered females (table 2*a*; figure 1). No control or cross-fostered females helped when the primary female died and was replaced by another female (figure 1). Cross-fostering, stage of experimentation, territory quality, group size, year of breeding and the continued presence of the primary male were not included in the final model (table 2*a*). The model was then repeated using the subset of data for which the additional variable—subordinate–primary female relatedness—was known. In this analysis, the continued presence of the primary female remained significant, whereas none of the other variables, including subordinate–primary female relatedness, helped to explain the variation in helping decisions (table 2*b*).

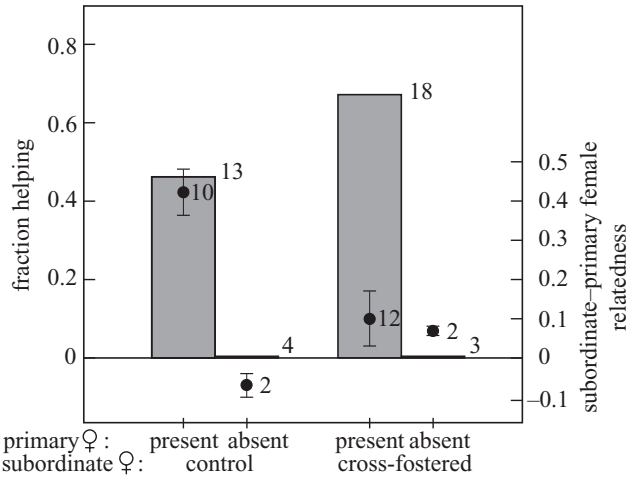

Figure 1. The fractions (grey bars) of control and cross-fostered female subordinate Seychelles warblers that helped to feed nestlings during their second year of life in relation to the continued presence of the primary females and primary foster females ($\chi^2 = 1.30$, d.f. = 1, $p = 0.253$) and in relation to the new primary females that replaced the primary (foster) females that raised the subordinates (absent). Only those subordinates that could have been helpers, because nests with young were present in their natal territory, were included ($n = 38$ individuals). Circles indicate mean relatedness of subordinates (±s.e.) to either primary females and primary foster females that raised the subordinates and were still present on the territory (present; $t = 3.48$, d.f. = 20, $p = 0.002$) or to new primary females that replaced the primary (foster) females that raised the subordinates (absent). The numbers of subordinates are indicated (1995–2001; see also table 2).

We were unable to relate helping decisions by female subordinates during their third year of age to association or genetic relatedness, as only 32 survived to the third year, and only six of these (four control and two cross-fostered young: 19%) remained on their natal territory.

4. DISCUSSION

(a) *Cue for helping behaviour*

In this study we concentrated on female subordinates, as in the Seychelles warbler most helping subordinates are female (88%; Komdeur 1996*a*, and 90%; this study), and only female subordinates appear to adjust provisioning according to relatedness (Richardson *et al.* 2003*a*,*b*). Helping by female non-parent subordinates is strongly correlated with kinship (Komdeur 1994*a*), with the probability of helping increasing with higher subordinate–nestling genetic relatedness (Richardson *et al.* 2003*a*,*b*). A process in which the recognition cues of probable relatives are learned is the likely mechanism for the expression of

kin preferences in birds (Sherman *et al.* 1997; Price 1998, 1999; Komdeur & Hatchwell 1999; Russell & Hatchwell 2001) and mammals (Clarke & Faulkes 1999). However, self-referent phenotype matching, a genetic-relatedness cue not achieved through learning, has recently been invoked to explain kin discrimination in several avian and mammalian species (Petrie *et al.* 1999; Hauber & Sherman 2001, 2003). In the Seychelles warbler, kin discrimination by subordinate females appears to be based not on direct measurements of genetic relatedness, but on associative-learning cues (Komdeur 1994*a*), i.e. the continued presence of the primary female (Richardson *et al.* 2003*a*,*b*). However, experiments are required to unravel the two mechanisms of kin recognition adequately (Komdeur & Hatchwell 1999; Russell & Hatchwell 2001).

In the present study we used an experimental design to investigate the cue used by offspring to determine when to remain on a territory as a subordinate and help feed the nestling. Cross-fostering of eggs and nestlings resulted in cross-fostered offspring that were not related to the primary female that raised them. Relatedness between nestlings and the primary female birds in the control group remained high. The critical requirement for testing the kin-discrimination cues used by subordinates is that offspring from experimental nests treated their foster parents as true parents when making cooperative breeding (staying and helping) decisions (Hatchwell *et al.* 2001). In this study we appear to have been able to keep the strength of the recognition template the same for cross-fostered and control nestlings.

(i) Nestlings hatched from cross-fostered eggs were not imprinted on the feeders before cross-fostering.
(ii) Cross-fostered nestlings were unlikely to be imprinted on the feeders before cross-fostering, as this was performed soon after hatching. Moreover, the long period of parental care (three months; Komdeur 1996*b*) and residency on the natal territory (at least nine months; Komdeur 1992, 1996*a*) provides a long learning period during which the recognition template can be established.
(iii) Foster parents did not discriminate between their own and fostered nestlings (this study).

Our cross-fostering experiment showed that female subordinates base their helping decisions on associative-learning cues, not on genetic-relatedness cues.

(i) Female subordinate helpers on experimental territories did not discriminate (in terms of food provisioning) between the non-fostered nestling before the swap and the cross-fostered nestling after the swap. This provides evidence that they were not directly assessing their relatedness to the nestlings. This result concurs with abundant evidence from birds in general, showing that individuals do not discriminate related from unrelated nestlings (Kempenaers & Sheldon 1996).
(ii) Cross-fostered subordinates regard foster parents as true parents. The proportions of cross-fostered and control offspring that became subordinate helpers on their 'natal' territory were similar, despite the fact that cross-fostered offspring were not related to other adults in their territory. Hence subordinates were not assessing their relatedness to other adult group members directly.

(**b**) *Alternative interpretations*

We have interpreted our results as being evidence for an associative-learning rule for helping decisions, as we are able to rule out the alternative explanations. For instance, we can dismiss the possibilities that potential helpers use spatial cues to recognize kin when making helping decisions (Hepper 1991; Komdeur & Hatchwell 1999) and that they are physiologically primed to carry out parent-like activities (*sensu* Jamieson 1989) for the following reasons. For both cross-fostered and control offspring, the chance of becoming a subordinate helper was significantly associated with the continued presence of the primary female but not with any of the other factors tested. If the primary female was absent, the offspring did not become subordinate helpers even if they remained on the territory that they perceived to be their natal territory. In fact, spatial cues would provide unreliable information on kinship as, occasionally, there can be considerable turnover of primary breeders, e.g. owing to high mortality caused by severe weather events (D. S. Richardson, unpublished data).

Helping by subordinates is also determined by whether or not they gain parentage within the breeding group, as Richardson *et al.* (2003*a*,*b*) showed that subordinate parents always provisioned. Ideally, we would have been able to distinguish between subordinates that did gain parentage within the group and those that did not. However, we have strong reasons to believe that subordinate parentage had no effect on our results. First, female subordinate parentage among the female subordinates used in this study during the second year of life (25%, $n = 16$; D. S. Richardson, unpublished data) is lower than in subsequent years (44%; Richardson *et al.* 2001) and here we restrict our analysis to this age group of birds. Second, the chance that a subordinate has gained parentage is unlikely to differ between cross-fostered and control subordinate females, as the gaining of parentage by a subordinate is not affected by her relatedness to the primary female (Richardson *et al.* 2002). In our study the chances of a subordinate gaining parentage during the second year of life were equal for cross-fostered and control females (cross-fostered: 28.6% (two out of seven), and control: 22.2 (two out of nine); $\chi^2 = 0.09$, d.f. = 1, $p = 0.77$).

There are limitations to the effectiveness of associative learning as a kin-recognition mechanism because of its vulnerability to errors resulting from accidental association, brood parasitism or extra-pair paternity (Komdeur & Hatchwell 1999). Any non-kin present in the right place during the 'associative-learning period' will be regarded as kin, whereas true kin who are absent will be regarded as non-kin. This has been shown to be the case in the Seychelles warbler. When a subordinate had a choice of helping at either of two different nests at the same time on its natal territory, it did not always feed the nestling produced by the breeders to whom it was most related; instead it provisioned only at those nests with breeders that had fed it as a nestling (Komdeur 1994*a*).

In societies where multiple females lay eggs in the same nest and care for the brood, such as in the Seychelles warbler (Richardson *et al.* 2002), it is unlikely that potential helpers can effectively discriminate between their genetic mother and any other female care provider. In the present study, a substantial number of the nestlings were raised by both a primary and a subordinate female simultaneously (39.4%, 13 out of 33). In the Seychelles warbler this problem is mitigated by the fact that primary female and subordinate females are normally closely related (mother and daughter); therefore potential helpers will be related to subsequent offspring of either female.

Finally, high levels of extra-pair or extra-group paternity may reduce the relatedness between a potential helper and the subsequent offspring produced by the recognized kin. Seychelles warblers avoid helping at a nest where they are not at all related by basing their associative-learning cue on the female carers that helped them as a nestling and not on the male carer (who may not actually be their father; Richardson *et al.* 2003*a*,*b*).

A simple learning rule, based on recognition through association, will be confounded by the factors (accidental association, brood parasitism and extra-pair paternity) listed above, and helpers will not be able to discriminate between close kin, more distantly related kin and unrelated individuals at the same nest. However, such a rule can still be kin-selected for as long as the mean relatedness of the recipients is sufficiently high to satisfy Hamilton's (1964) rule (Keller 1997). In the Seychelles warbler this appears to be the case as, on average, female subordinate helpers are significantly related to the offspring they help (Richardson *et al.* 2003*a*,*b*).

(c) *Associative-learning period and cues*

Recognition by associative learning requires that the animal learns the cues that identify its putative kin and then uses those cues to recognize its kin outside the association context (Komdeur & Hatchwell 1999). This implies a learning period during which the recognition template is established (Sherman *et al.* 1997; Price 1998, 1999; Komdeur & Hatchwell 1999). There are several experimental studies of kin recognition based on association that have investigated the imprinting period. In mammals the short period (hours) immediately after birth is important for the development of kin recognition (e.g. Holmes & Sherman 1982; Robinson & Smotherman 1991; Holmes 2003). Kin recognition in mammals might already have developed in the uterine environment (Robinson & Smotherman 1991). To our knowledge, in birds the only study of kin recognition based on association that has investigated the imprinting period is that of the long-tailed tit. In this species, young birds learn the vocalizations of kin and non-kin during the period of care (Hatchwell *et al.* 2001), and these are used as a recognition and discrimination cue for helping decisions in later years (Russell & Hatchwell 2001). The use of vocalization cues as a recognition mechanism has been studied in two other cooperatively breeding bird species. Stripe-backed wrens, *Campylorhynchus nuchalis*, discriminated between the calls of different patrilines, rather than on the basis of group membership (Price 1999). Conversely, splendid fairy-wrens, *Malurus splendens,* did not discriminate between the songs of group and non-group members (Payne *et al.* 1988). Interesting avenues of investigation for birds using a discrimination mechanism based on association are the determination of the associative-learning period and the cue(s) used for association.

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REFERENCES

Arnold, W. 1990 The evolution of marmot sociality. 2. Costs and benefits of joint hibernation. *Behav. Ecol. Sociobiol.* **27**, 239–246.

Blaustein, A. R., Bekoff, M. & Daniels, T. J. 1987 Kin recognition in vertebrates (excluding primates): empirical evidence. In *Kin recognition in animals* (ed. D. J. C. Fletcher & C. D. Michener), pp. 287–331. Chichester, UK: Wiley.

Brown, J. L. 1987 *Helping and communal breeding in birds*. Princeton University Press.

Clarke, F. M. & Faulkes, C. G. 1999 Kin discrimination and female mate choice in the naked mole-rat *Heterocephalus glaber*. *Proc. R. Soc. Lond.* B **266**, 1995–2002. (DOI 10.1098/rspb.1999.0877.)

Clarke, M. F. 1984 Cooperative breeding by the Australian bell miner, *Manorina melanophrys* Latham: a test of kin selection theory. *Behav. Ecol. Sociobiol.* **14**, 137–146.

Clutton-Brock, T. H., Botherton, P. N. M., O'Riain, M. J., Griffin, A. S., Gaynor, D., Sharpe, L., Kansky, R., Manser, M. B. & McIlrath, G. M. 2000 Individual contributions to babysitting in a cooperative mongoose, *Suricata suricatta*. *Proc. R. Soc. Lond.* B **267**, 301–305. (DOI 10.1098/rspb.2000.1000.)

Clutton-Brock, T. H., Botherton, P. N. M., O'Riain, M. J., Griffin, A. S., Gaynor, D., Kansky, R., Sharpe, L., Manser, M. B. & McIlrath, G. M. 2001 Contributions to cooperative rearing in meerkats *Suricata suricatta*. *Anim. Behav.* **61**, 705–710.

Cockburn, A. 1998 Evolution of helping behaviour in cooperatively breeding birds. *A. Rev. Ecol. Syst.* **29**, 141–177.

Curry, R. L. 1988 Influence of kinship on helping behavior in Galapagos mockingbirds. *Behav. Ecol. Sociobiol.* **22**, 141–152.

Emlen, S. T. 1997 Predicting family dynamics in social vertebrates. In *Behavioural ecology: an evolutionary approach* (ed. J. R. Krebs & N. B. Davies), pp. 228–253. Oxford: Blackwell.

Emlen, S. T. & Wrege, P. H. 1988 The role of kinship in helping decisions among white-fronted bee-eaters. *Behav. Ecol. Sociobiol.* **23**, 305–315.

Griffin, A. S. & West, S. A. 2003 Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. *Science* **302**, 634–636.

Griffiths, R., Double, M. C., Orr, K. & Dawson, R. J. G. 1998 A DNA test to sex most birds. *Mol. Ecol.* **7**, 1071–1075.

Hamilton, W. D. 1964 The evolution of altruistic behavior. *Am. Nat.* **97**, 354–356.

Hatchwell, B. J., Ross, D. J., Fowlie, M. K. & McGowan, A. 2001 Kin discrimination in cooperatively breeding long-tailed tits. *Proc. R. Soc. Lond.* B **268**, 885–890. (DOI 10.1098/rspb.2001.1598.)

Hauber, M. E. & Sherman, P. W. 2001 Self-referent phenotype matching: theoretical considerations and empirical evidence. *Trends Neurosci.* **24**, 609–616.

Hauber, M. E. & Sherman, P. W. 2003 Designing and interpreting experimental tests of self-referent phenotype matching. *Anim. Cogn* **6**, 69–71.

Hepper, P. G. 1991 *Kin recognition*. Cambridge University Press.

Holmes, W. G. 2003 The development and function of nepotism. Why kinship matters in social relationships. In *Handbook of behavioral neurobiology series*, vol. 13 (ed. E. M. Blass), pp. 281–316. Dordrecht, The Netherlands: Kluwer Academic.

Holmes, W. G. & Sherman, P. W. 1982 The ontogeny of kin recognition in two species of ground squirrels. *Am. Zool.* **22**, 491–519.

Jamieson, I. G. 1989 Behavioral heterochrony and the evolution of birds' helping at the nest: an unselected consequence of communal breeding? *Am. Nat.* **133**, 394–406.

Keller, L. 1997 Indiscriminate altruism: unduly nice parents and siblings. *Trends Ecol. Evol.* **12**, 99–103.

Kempenaers, B. & Sheldon, B. C. 1996 Why do male birds not discriminate between their own and extra-pair offspring? *Anim. Behav.* **51**, 1165–1173.

Komdeur, J. 1991 Cooperative breeding in the Seychelles warbler. PhD thesis, University of Cambridge, UK.

Komdeur, J. 1992 Importance of habitat saturation and territory quality for evolution of cooperative breeding in the Seychelles warbler. *Nature* **358**, 493–495.

Komdeur, J. 1994*a* The effect of kinship on helping in the cooperative breeding Seychelles warbler (*Acrocephalus sechellensis*). *Proc. R. Soc. Lond.* B **256**, 47–52.

Komdeur, J. 1994*b* Experimental evidence for helping and hindering by previous offspring in the cooperative breeding Seychelles warbler. *Behav. Ecol. Sociobiol.* **34**, 175–186.

Komdeur, J. 1996*a* Facultative sex ratio bias in the offspring of Seychelles warblers. *Proc. R. Soc. Lond.* B **263**, 661–666.

Komdeur, J. 1996*b* Seasonal timing of reproduction in a tropical bird, the Seychelles warbler: a field experiment using translocation. *J. Biol. Rhythms* **11**, 333–346.

Komdeur, J. 1999 Reproductive control in cooperatively and polygynously breeding *Acrocephalus* species. In *Proc. 22 Int. Ornithol. Congr., Durban, Univ. Natal* (ed. N. Adams & R. Slotow), pp. 2910–2921. Johannesburg: BirdLife South Africa.

Komdeur, J. & Hatchwell, B. J. 1999 Kin recognition: function and mechanism in avian societies. *Trends Ecol. Evol.* **14**, 237–241.

Komdeur, J. & Kats, K. H. 1999 Predation risk affects trade-off between nest guarding and foraging in Seychelles warblers. *Behav. Ecol.* **10**, 648–658.

Maynard Smith, J. 1964 Group selection and kin selection. *Nature* **201**, 1145–1147.

Payne, R. B., Payne, L. L. & Rowley, I. 1988 Kin and social relationships in splendid fairy-wrens: recognition by song in a cooperative bird. *Anim. Behav.* **36**, 1341–1351.

Petrie, M., Krupa, A. & Burke, T. 1999 Peacocks lek with relatives, even in the absence of social or environmental cues. *Nature* **401**, 155–157.

Price, J. J. 1998 Family- and sex-specific vocal traditions in a cooperatively breeding songbird. *Proc. R. Soc. Lond.* B **265**, 497–502. (DOI 10.1098/rspb.1998.0322.)

Price, J. J. 1999 Recognition of family-specific calls in stripe-backed wrens. *Anim. Behav.* **57**, 483–492.

Reyer, H.-U. 1984 Investment and relatedness: a cost/benefit analysis of breeding and helping in the pied kingfisher (*Ceryle rudis*). *Anim. Behav.* **32**, 1163–1178.

Richardson, D. S., Jury, F. L., Dawson, D., Salgueiro, P., Komdeur, J. & Burke, T. 2000 Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Mol. Ecol.* **9**, 2226–2231.

Richardson, D. S., Jury, F. L., Blaakmeer, K., Komdeur, J. & Burke, T. 2001 Parentage assignment and extra-group paternity in a cooperative breeder: the Seychelles warbler (*Acrocephalus sechellensis*). *Mol. Ecol.* **10**, 2263–2273.

Richardson, D. S., Burke, T. & Komdeur, J. 2002 Direct benefits and the evolution of female-biased cooperative breeding in Seychelles warblers. *Evolution* **56**, 2313–2321.

Richardson, D. S., Komdeur, J. & Burke, T. 2003*a* Altruism and infidelity among warblers. *Nature* **422**, 580.

Richardson, D. S., Burke, T. & Komdeur, J. 2003*b* Sex-specific associative learning cues and inclusive fitness benefits in the Seychelles warbler. *J. Evol. Biol.* **16**, 854–861.

Robinson, S. C. & Smotherman, W. P. 1991 Fetal learning: implications for the development of kin recognition. In *Kin recognition* (ed. P. G. Hepper), pp. 308–334. Cambridge University Press.

Russell, A. F. 1999 Ecological constraints and the cooperative breeding system of the long-tailed tit *Aegithalos caudatus*. PhD thesis, University of Sheffield, UK.

Russell, A. F. & Hatchwell, B. J. 2001 Experimental evidence for kin-biased helping in a cooperatively breeding vertebrate. *Proc. R. Soc. Lond.* B **268**, 2169–2174. (DOI 10.1098/rspb.2001.1790.)

Sherman, P. W., Reeve, H. K. & Pfennig, D. W. 1997 Recognition systems. In *Behavioural ecology: an evolutionary approach* (ed. J. R. Krebs & N. B. Davies), pp. 69–96. Oxford: Blackwell.

SPSS 2001 *SPSS 11.0*. Chicago, IL: SPSS Inc.

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